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Chitinase determinants of Vibrio vulnificus: gene cloning and applications of a chitinase probe.

Wortman AT, Somerville CC, Colwell RR.

To initiate study of the genetic control of chitinolytic activity in vibrios, the chitobiase gene was isolated by cloning chromosomal DNA prepared from Vibrio vulnificus. Chimeric plasmids were constructed from Sau3A I partial digests of chromosomal DNA by ligating 5 to 15-kilobase fragments into the BamHI site, i.e., in the Tcr gene, of pBR322 (Amr Tcr). The resulting plasmids were transformed into Escherichia coli DH1. Chitobiase activity of the insert-bearing clones was detected by using a chromogenic substrate, p-nitrophenyl-N-acetyl-beta, D-glucosaminide, and confirmed by the appearance of a fluorescent end product from the hydrolysis of 4-methylumbelliferyl-beta,D-N-N'-diacetylchitobiose. Endochitinase activity was demonstrated by liberation of water-soluble products produced by the degradation of [3H]chitin. Transformation of E. coli Y10R (lacY) with plasmids from chitinase-positive clones restored the lactose-positive phenotype, suggesting the presence of a permease associated with chitinase activity. Physical mapping of plasmids containing the chitinase determinants indicate that transcription of these genes in E. coli may be initiated at a V. vulnificus promoter.

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